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GFP-LCA (wt)

FIG. 1



GFP-TLCA

FIG. 2

1	<u>P</u> <u>F</u> <u>U</u> <u>N</u> <u>K</u> <u>Q</u> <u>E</u> <u>N</u> <u>Y</u> <u>K</u>	<u>D</u> <u>P</u> <u>V</u> <u>N</u> <u>G</u> <u>V</u> <u>D</u> <u>I</u> <u>A</u> <u>Y</u>	<u>I</u> <u>K</u> <u>I</u> <u>P</u> <u>N</u> <u>V</u> <u>G</u> <u>Q</u> <u>M</u> <u>Q</u>	<u>P</u> <u>V</u> <u>K</u> <u>A</u> <u>F</u> <u>K</u> <u>I</u> <u>H</u> <u>N</u> <u>K</u>	<u>I</u> <u>W</u> <u>V</u> <u>I</u> <u>P</u> <u>E</u> <u>R</u> <u>D</u> <u>T</u> <u>F</u>
51	<u>T</u> <u>N</u> <u>P</u> <u>E</u> <u>E</u> <u>G</u> <u>D</u> <u>L</u> <u>N</u> <u>P</u>	<u>P</u> <u>P</u> <u>E</u> <u>A</u> <u>K</u> <u>Q</u> <u>V</u> <u>P</u> <u>V</u> <u>S</u>	<u>Y</u> <u>D</u> <u>S</u> <u>T</u> <u>Y</u> <u>L</u> <u>S</u> <u>T</u> <u>D</u>	<u>N</u> <u>E</u> <u>K</u> <u>D</u> <u>N</u> <u>Y</u> <u>L</u> <u>K</u> <u>G</u> <u>V</u>	<u>T</u> <u>K</u> <u>L</u> <u>F</u> <u>E</u> <u>R</u> <u>I</u> <u>Y</u> <u>S</u> <u>T</u>
101	<u>D</u> <u>L</u> <u>G</u> <u>R</u> <u>M</u> <u>L</u> <u>L</u> <u>T</u> <u>S</u> <u>I</u>	<u>V</u> <u>R</u> <u>G</u> <u>I</u> <u>P</u> <u>F</u> <u>W</u> <u>G</u> <u>G</u>	<u>T</u> <u>I</u> <u>D</u> <u>T</u> <u>E</u> <u>L</u> <u>K</u> <u>V</u> <u>I</u> <u>D</u>	<u>T</u> <u>N</u> <u>C</u> <u>I</u> <u>N</u> <u>V</u> <u>I</u> <u>Q</u> <u>P</u> <u>D</u>	<u>G</u> <u>S</u> <u>Y</u> <u>R</u> <u>S</u> <u>E</u> <u>E</u> <u>L</u> <u>N</u> <u>L</u>
151	<u>V</u> <u>I</u> <u>I</u> <u>G</u> <u>P</u> <u>S</u> <u>A</u> <u>D</u> <u>I</u> <u>I</u>	<u>Q</u> <u>F</u> <u>E</u> <u>C</u> <u>K</u> <u>S</u> <u>F</u> <u>G</u> <u>H</u> <u>E</u>	<u>V</u> <u>I</u> <u>N</u> <u>L</u> <u>T</u> <u>R</u> <u>N</u> <u>G</u> <u>G</u>	<u>S</u> <u>T</u> <u>Q</u> <u>Y</u> <u>I</u> <u>R</u> <u>F</u> <u>S</u> <u>P</u> <u>D</u>	<u>F</u> <u>T</u> <u>F</u> <u>G</u> <u>F</u> <u>E</u> <u>E</u> <u>S</u> <u>S</u> <u>E</u>
201	<u>V</u> <u>D</u> <u>T</u> <u>N</u> <u>P</u> <u>L</u> <u>L</u> <u>G</u> <u>A</u> <u>G</u>	<u>K</u> <u>F</u> <u>A</u> <u>T</u> <u>D</u> <u>P</u> <u>A</u> <u>V</u> <u>T</u> <u>L</u>	<u>A</u> <u>H</u> <u>E</u> <u>L</u> <u>I</u> <u>H</u> <u>A</u> <u>G</u> <u>H</u> <u>R</u>	<u>L</u> <u>G</u> <u>I</u> <u>A</u> <u>I</u> <u>N</u> <u>P</u> <u>N</u> <u>R</u>	<u>V</u> <u>F</u> <u>K</u> <u>V</u> <u>N</u> <u>T</u> <u>N</u> <u>A</u> <u>Y</u>
251	<u>E</u> <u>M</u> <u>S</u> <u>G</u> <u>L</u> <u>E</u> <u>V</u> <u>S</u> <u>F</u> <u>E</u>	<u>E</u> <u>L</u> <u>R</u> <u>T</u> <u>E</u> <u>G</u> <u>G</u> <u>H</u> <u>D</u> <u>A</u>	<u>K</u> <u>F</u> <u>I</u> <u>D</u> <u>S</u> <u>L</u> <u>Q</u> <u>E</u> <u>N</u> <u>E</u>	<u>E</u> <u>R</u> <u>L</u> <u>Y</u> <u>Y</u> <u>N</u> <u>K</u> <u>F</u> <u>K</u>	<u>D</u> <u>I</u> <u>A</u> <u>S</u> <u>T</u> <u>L</u> <u>N</u> <u>K</u>
301	<u>S</u> <u>I</u> <u>V</u> <u>G</u> <u>T</u> <u>A</u> <u>S</u> <u>I</u> <u>Q</u>	<u>Y</u> <u>M</u> <u>K</u> <u>N</u> <u>V</u> <u>E</u> <u>K</u> <u>E</u> <u>Y</u>	<u>L</u> <u>I</u> <u>S</u> <u>E</u> <u>D</u> <u>T</u> <u>S</u> <u>G</u> <u>K</u> <u>F</u>	<u>S</u> <u>V</u> <u>D</u> <u>K</u> <u>L</u> <u>K</u> <u>F</u> <u>D</u> <u>K</u> <u>L</u>	<u>Y</u> <u>K</u> <u>M</u> <u>L</u> <u>T</u> <u>E</u> <u>I</u> <u>Y</u> <u>T</u> <u>E</u>
351	<u>D</u> <u>N</u> <u>F</u> <u>V</u> <u>K</u> <u>F</u> <u>K</u> <u>V</u> <u>L</u>	<u>N</u> <u>R</u> <u>K</u> <u>T</u> <u>Y</u> <u>L</u> <u>N</u> <u>F</u> <u>D</u> <u>K</u>	<u>A</u> <u>V</u> <u>F</u> <u>K</u> <u>I</u> <u>N</u> <u>I</u> <u>V</u> <u>P</u> <u>K</u>	<u>V</u> <u>N</u> <u>Y</u> <u>T</u> <u>I</u> <u>D</u> <u>G</u> <u>F</u> <u>N</u>	<u>L</u> <u>R</u> <u>N</u> <u>T</u> <u>N</u> <u>L</u> <u>A</u> <u>N</u> <u>F</u>
401	<u>N</u> <u>G</u> <u>Q</u> <u>N</u> <u>T</u> <u>E</u> <u>I</u> <u>N</u> <u>M</u>	<u>N</u> <u>F</u> <u>T</u> <u>K</u> <u>L</u> <u>K</u> <u>N</u> <u>F</u> <u>T</u> <u>G</u>	<u>L</u> <u>F</u> <u>E</u> <u>F</u> <u>Y</u> <u>K</u> <u>L</u> <u>C</u> <u>V</u>	<u>R</u> <u>G</u> <u>I</u> <u>I</u> <u>T</u> <u>S</u> <u>K</u>	

FIG. 3

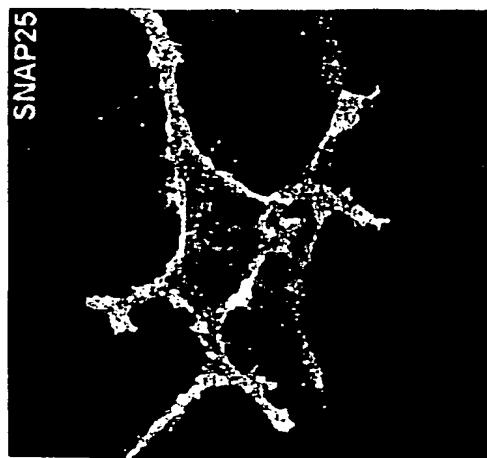


FIG. 5

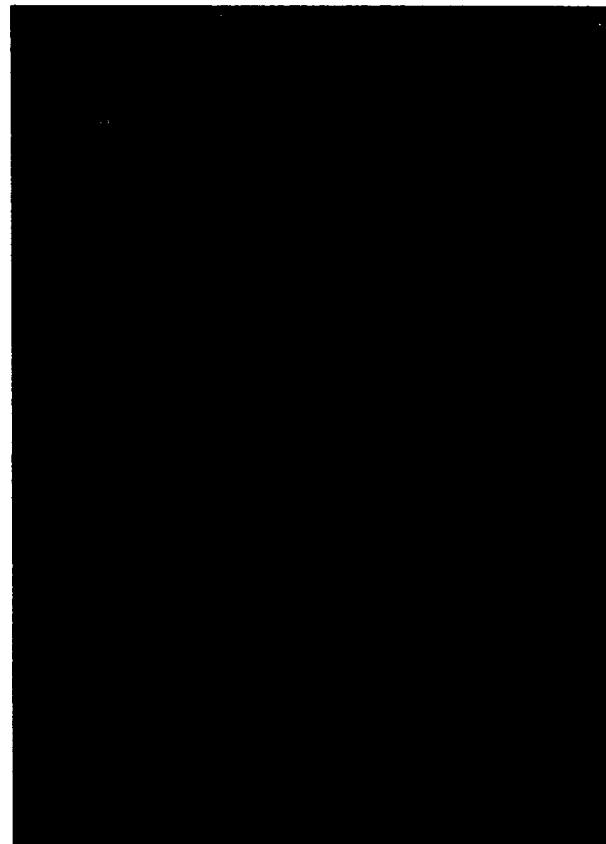
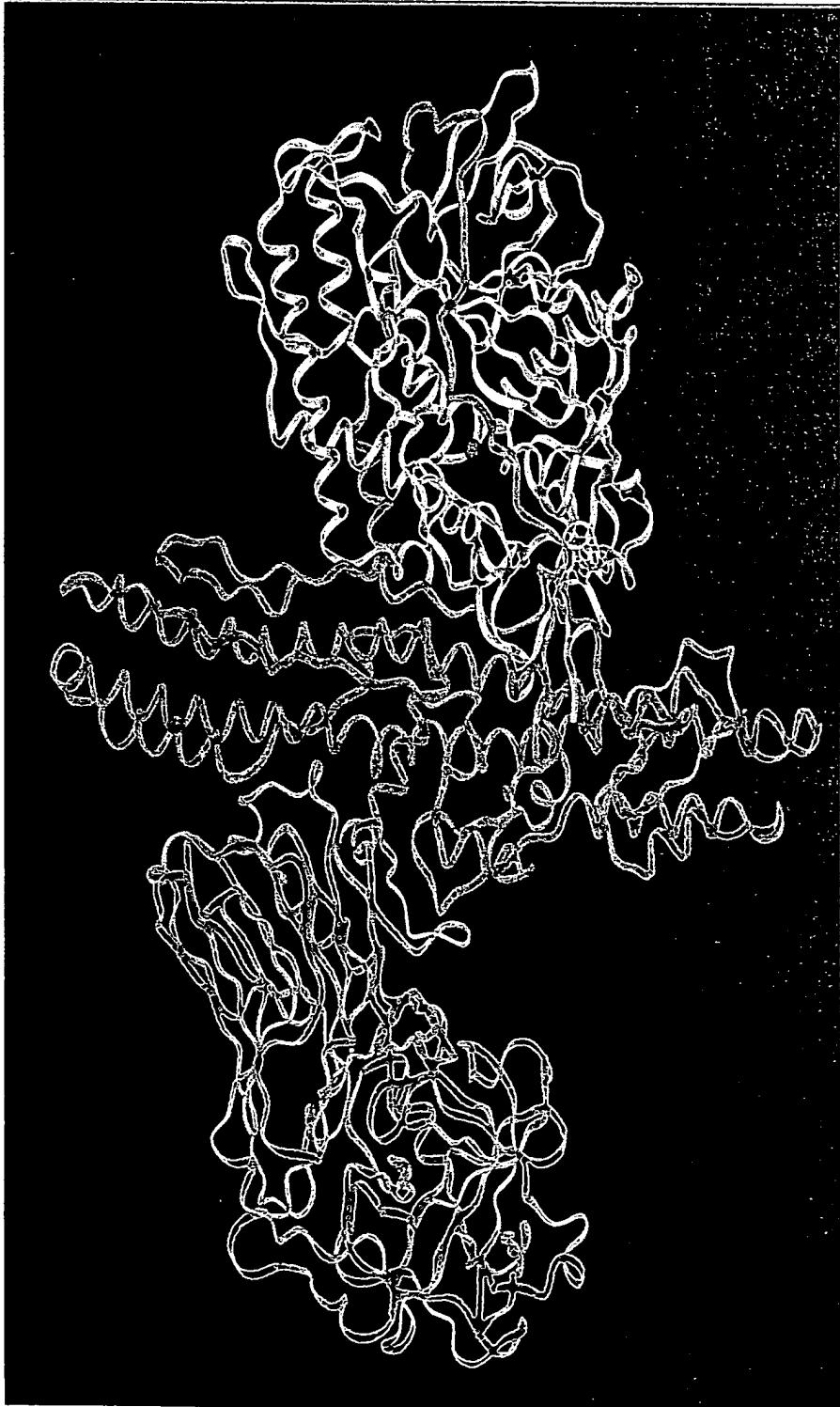


FIG. 4

Type A X-Ray Structure

Botulinum Toxin

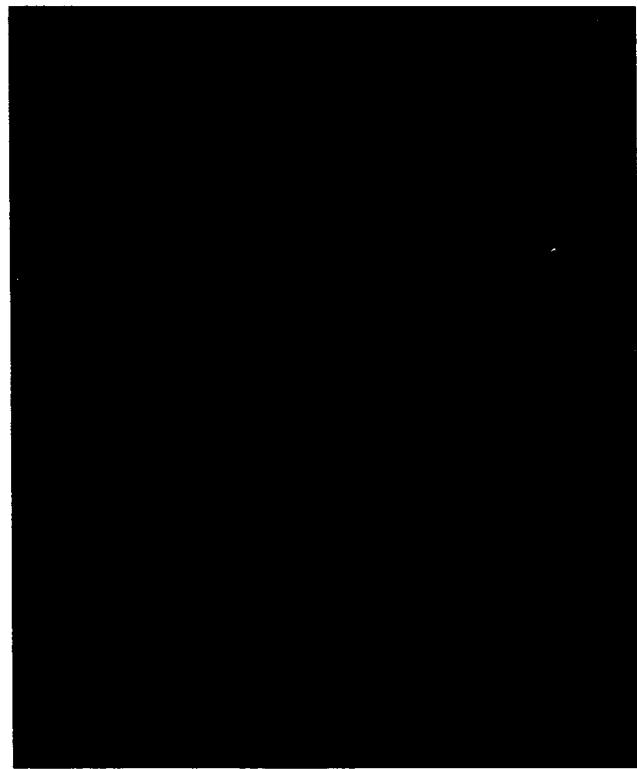


Yellow = protease domain

Green = binding domain

Red = translocational domain

FIG. 6



GFP-LCB (wt)

FIG. 7

1 BoNT-A_HalA_LC (1) - P[FV NK Q [FNY]K [DPV] N G V D [AY I K I] P[NV G - Q M Q P V K[A F K] I H N K [W] V [P E R] D [F T] N P E G D L N P P E A K Q V P V S Y D
 BoNT-B_Danish_L_LC (1) M [P V T I N N [FNY]N [D P I D N N N] I M M E P P F A R G T G R Y Y K[A F K] I D R [W] I [P E R] Y [E G Y K] E D F N K S S G I F N R D V C E Y Y D
 Consensus (1) P N F N Y D P I I I P K A F K I K I W I I P E R T F E

75
 76 BoNT-A_HalA_LC (74) S T Y [I] S [D N E K D N Y [K G V T K L F E R] Y S T D [G R M] [L T] S [I V R G] P E K [G S T] I D T [E L K V I D T N C I N V] I Q P D G S Y R - S [E P D Y [I N] N D K [K N] I F [Q T M] I K L E N [R K] S [K P] G E K [I E M] [I N] G [P Y] L [G D R R V] P L [E F N T N] I A S V T [V N K L I S N P G E V [E R
 BoNT-B_Danish_L_LC (76) Y L T K F L M K L F R I S L G [L L] I I G I P F G I E V
 Consensus (76)

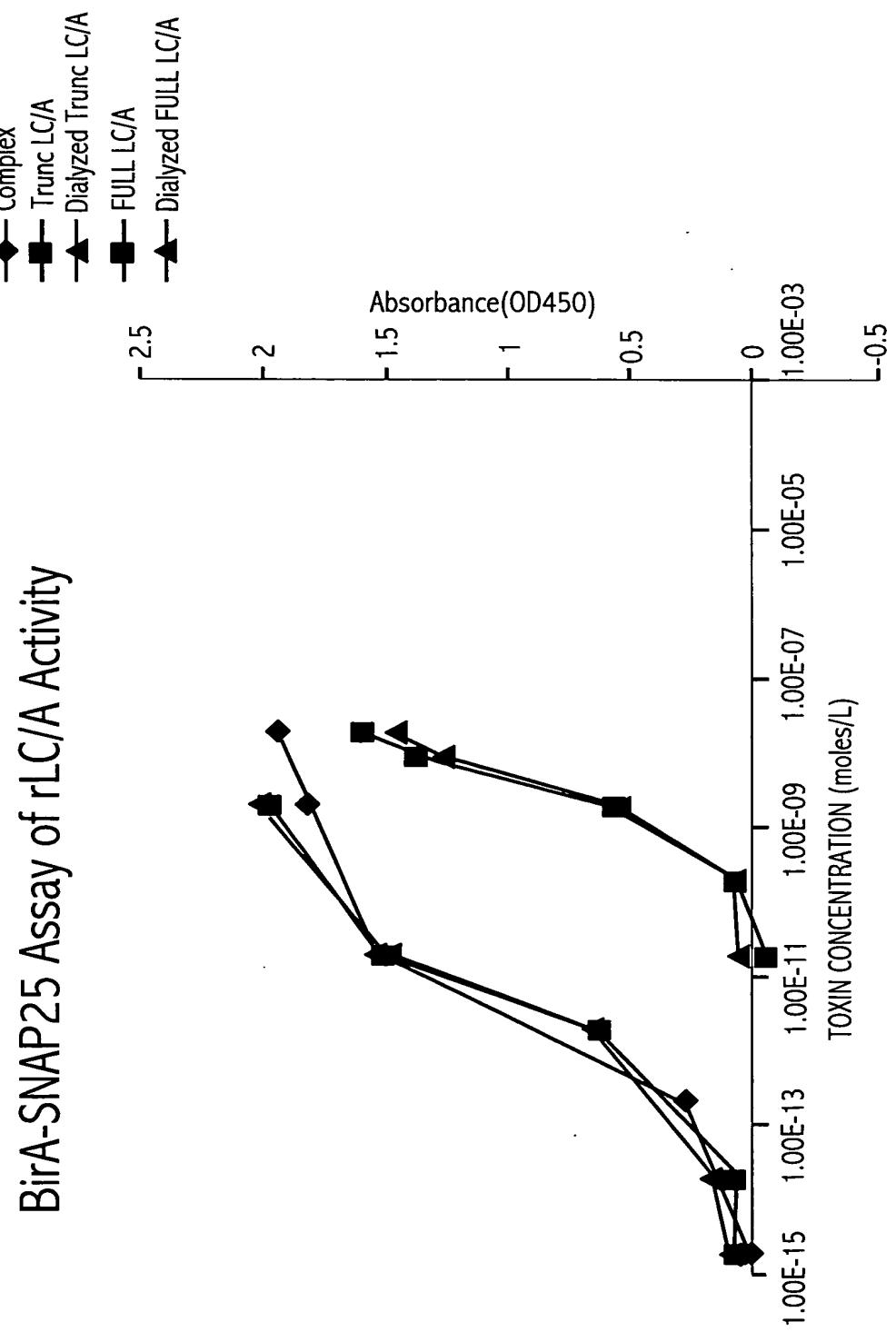
150
 151 BoNT-A_HalA_LC (148) . . . - L [N] [V] D [G P] S A D I Q F E C K S F [G] H E V L N L T [R N] G [S T] Q Y I R [F S P D] F T F G [F] E E S L [E V D T N P L L G A G K F A T [D P A
 BoNT-B_Danish_L_LC (151) K K G I F A [N] [I] D [G P] G P V L N E N [I] D I G I Q N H F A S [R E G] G [G I M] Q M K [E C P E Y] V S V [E N N V] Q [E N K G A S I F N R R G Y F S D P A
 Consensus (151) N L I I G P I E G S R G F G I K F P D F F E

225
 226 BoNT-A_HalA_LC (218) V T [I] A H E L I H A G [H R] L Y G] A L N P N R V F K V [N T N A Y Y E M S G L E V S F E E L R [T F G G] H D A K F [D D S L Q E N E F R L Y Y N K [E K D] I
 BoNT-B_Danish_L_LC (226) L I [Q] M H E L I H V L [H G] L Y G] K V D - D L P I V P N E K K F M Q [S T D A I Q A E E L Y T [T F G G] Q [D P S I I T P S T D K S I Y D K V L Q N [E R G] D
 Consensus (226) L L H E L I H H L Y G I I N N F F S I E E L T F G G D

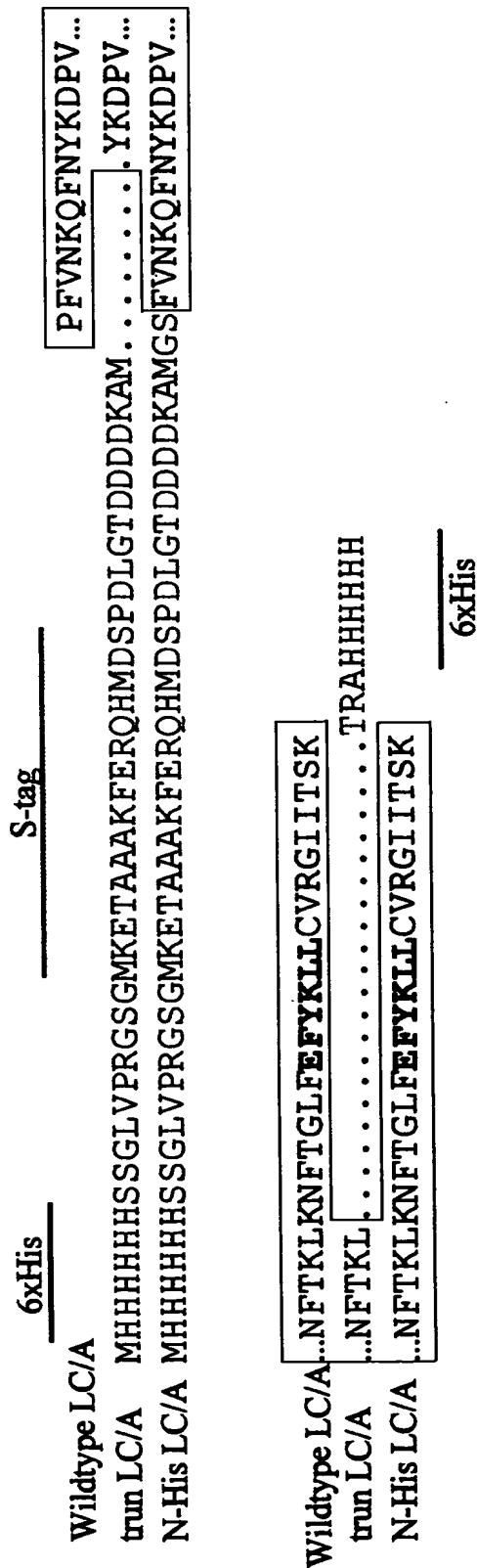
300
 301 BoNT-A_HalA_LC (293) A S T [I N] K A K S I V G - T T A S L Q Y M [K N V] E K E [K Y] L L S E D I S G K F F S V D [K L K] F D K L K Y K M [I T E Y I E D N] F V K F F K [V L N] R [K T] Y
 BoNT-B_Danish_L_LC (300) V D R [L N] K V L V C I S D P N I N I Y K [K N K] F K D [K Y] K F V [D S E G K Y] S [M F G F] E T N [A E N Y K] K T R A S Y F
 Consensus (301) L N K I I N K N F K D K Y E D S G K F S I D F D K L Y K L F T E N F X I R S Y

375
 376 BoNT-A_HalA_LC (367) N F D K A V F [K] N - I V P K V N [Y] D [G F N] L R N T N L A A N F N G Q N T E [N] M M N F T K L K N F T G L F E F [Y] K L L C V R G I I T S K
 BoNT-B_Danish_L_LC (375) S D S L P P V [K] K N L L D N E I Y T I E D [G F N] S D K D M E K E Y R G Q N K A [N K Q A Y E E I S - - K E H L A V [Y] K I Q M C K S V K - - -
 Consensus (376) K I L Y T I D G F N I L F G Q N I N F I

FIG. 8

**FIG. 9**

Comparison of LC/A constructs expressed from E. coli for in vitro analysis



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Truncated LC construct published

- Kadkhodayan, S. et al. Prot. Exp. Purif. 2000, 19, 125-130
- Crystal structure reported at IBRCC in Oct. 2000

FIG. 10



FIG. 11

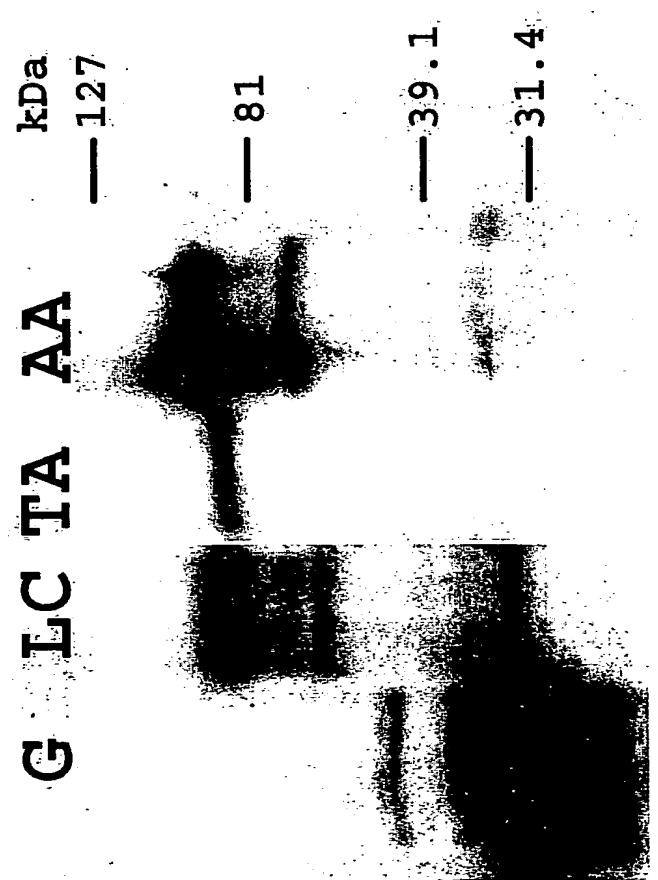


FIG. 12

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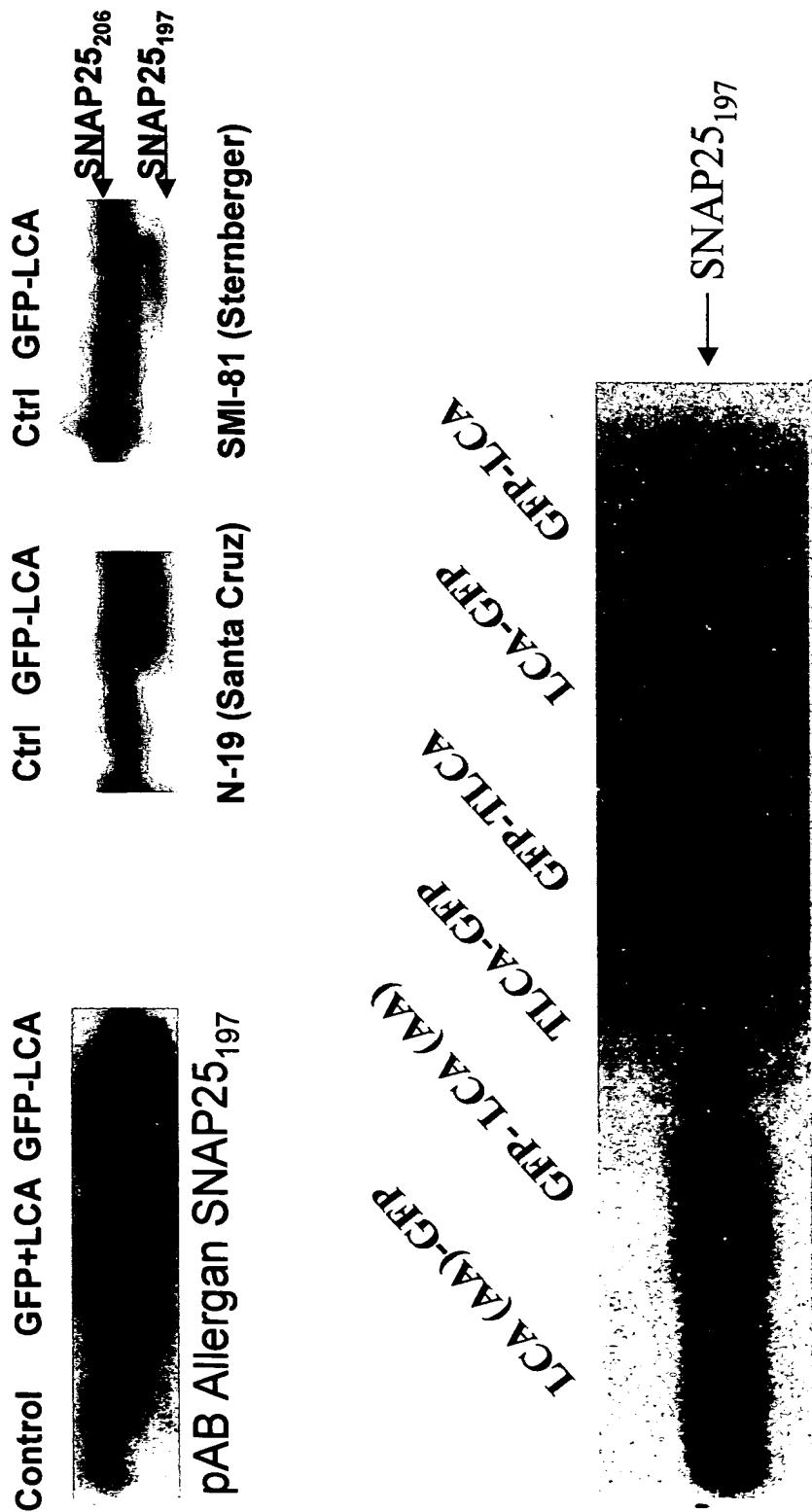


FIG. 13

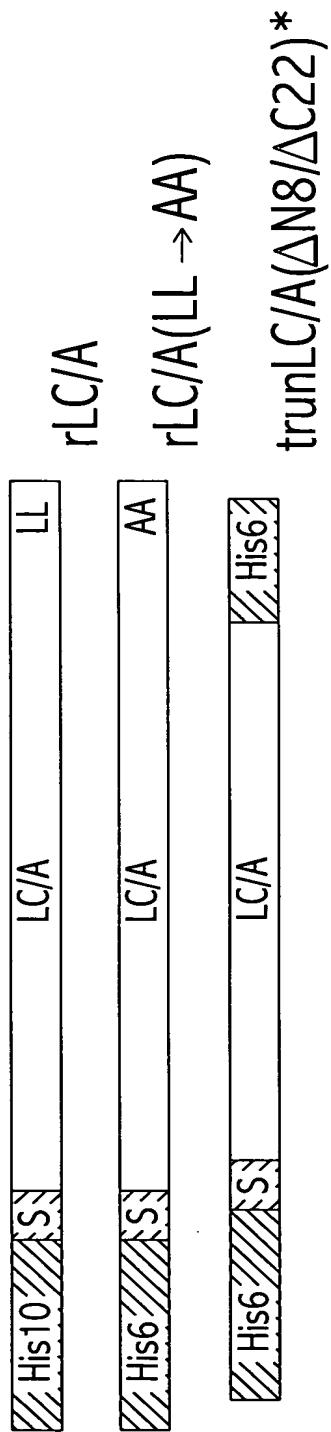
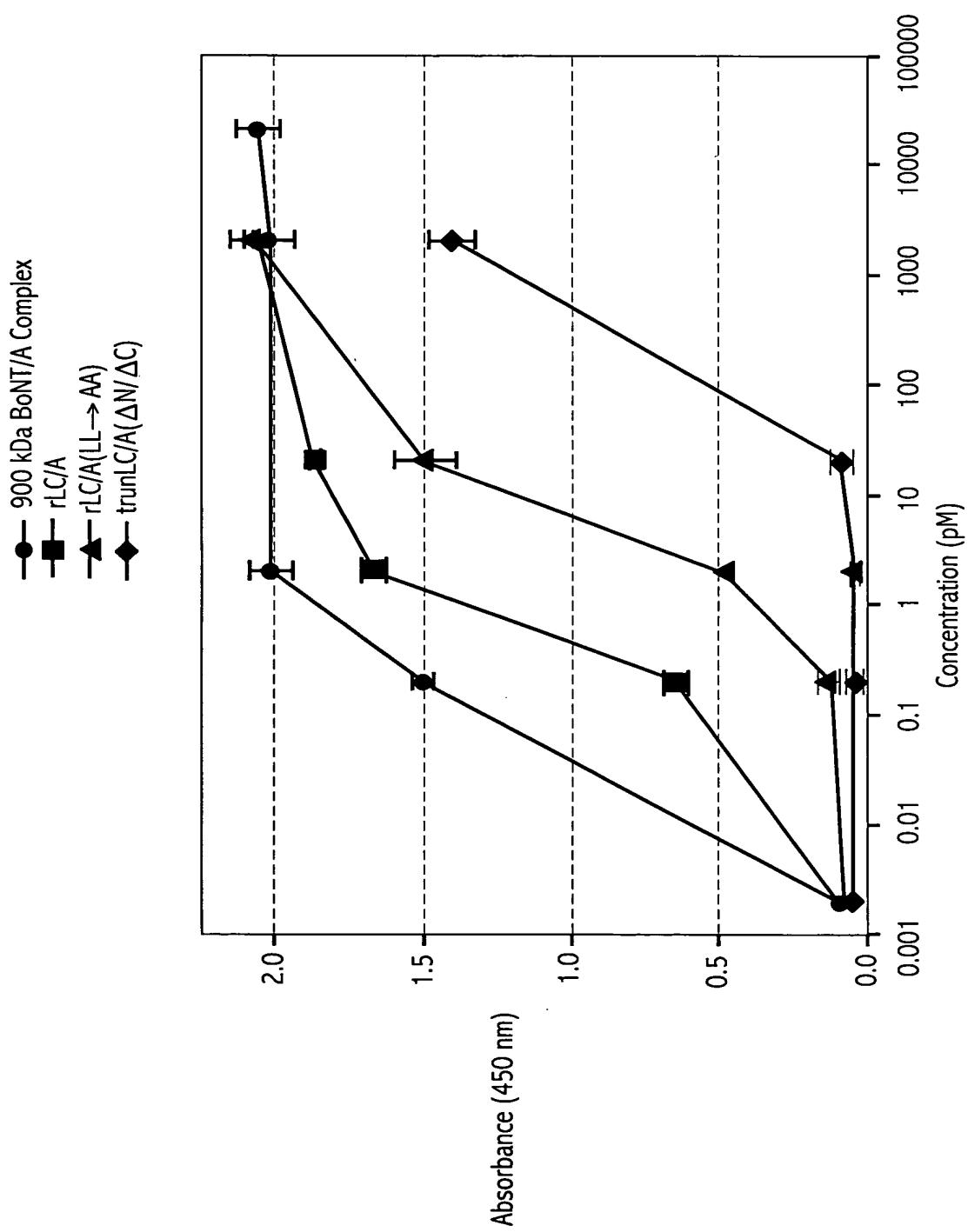


FIG. 14

**FIG. 15**

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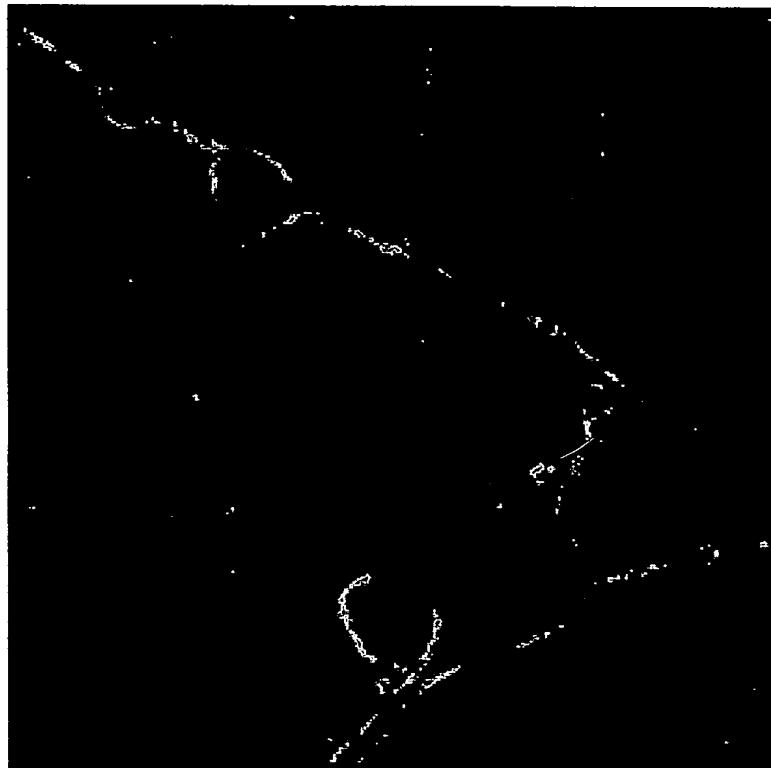


FIG. 16

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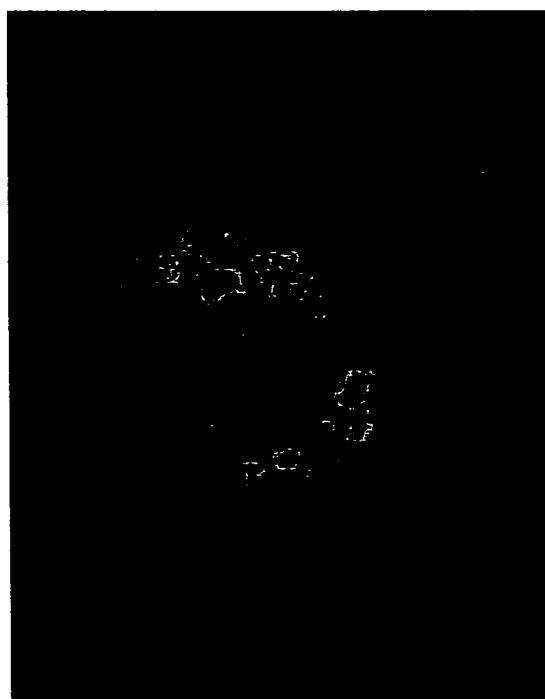
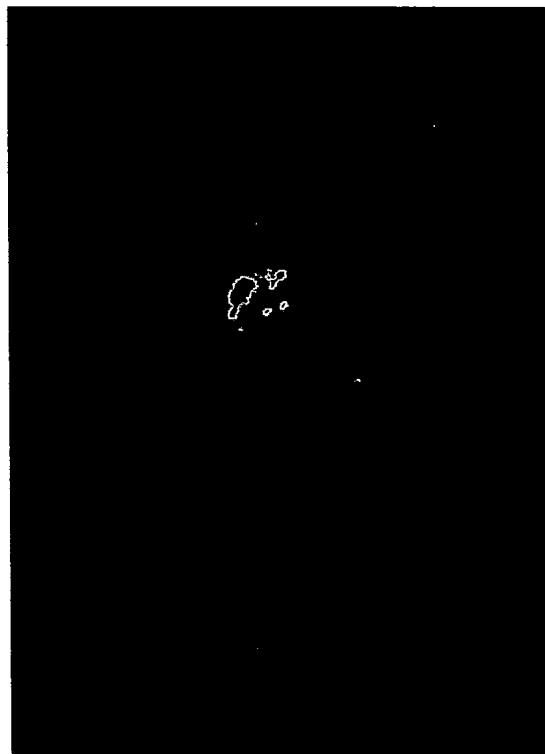


FIG. 17

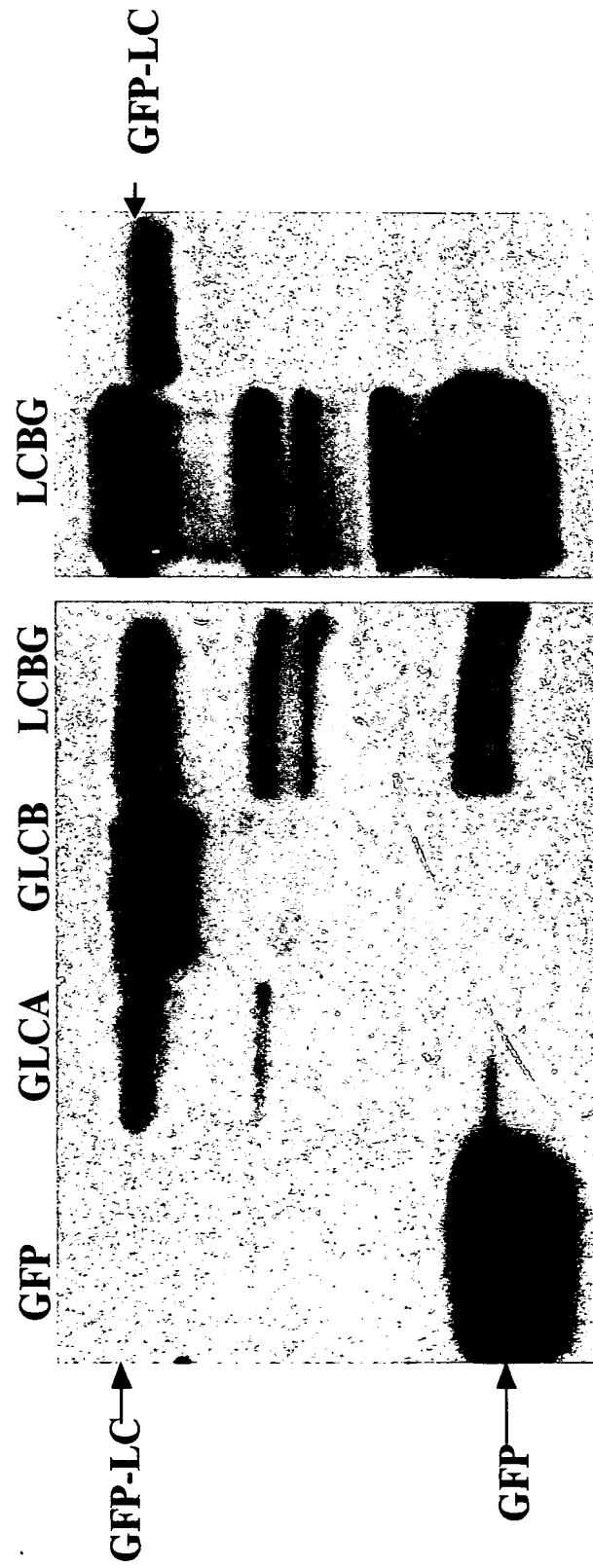
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FIG. 18



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IP: GFP (3E2) // WB:GFP (PolyAb)

FIG. 19

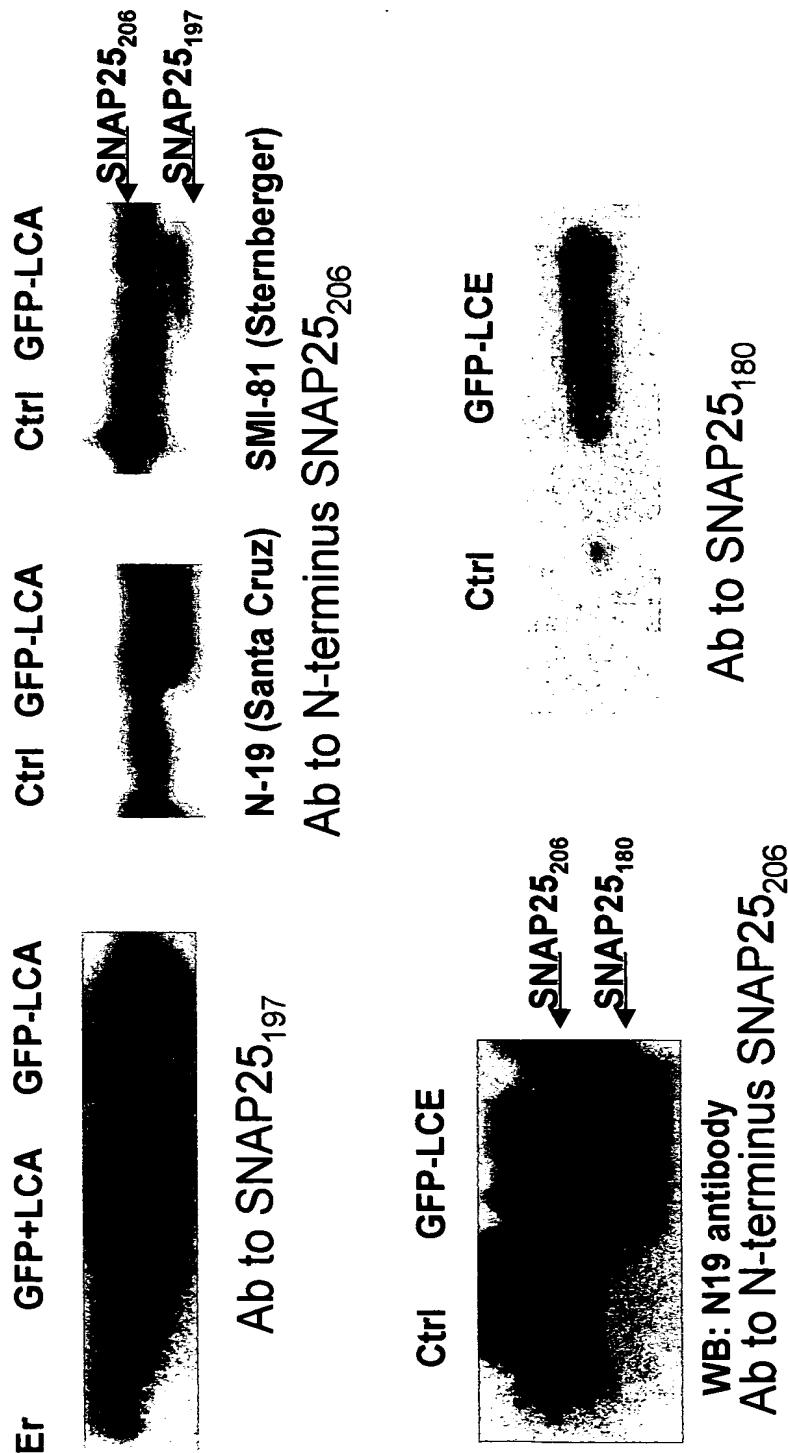


FIG. 20

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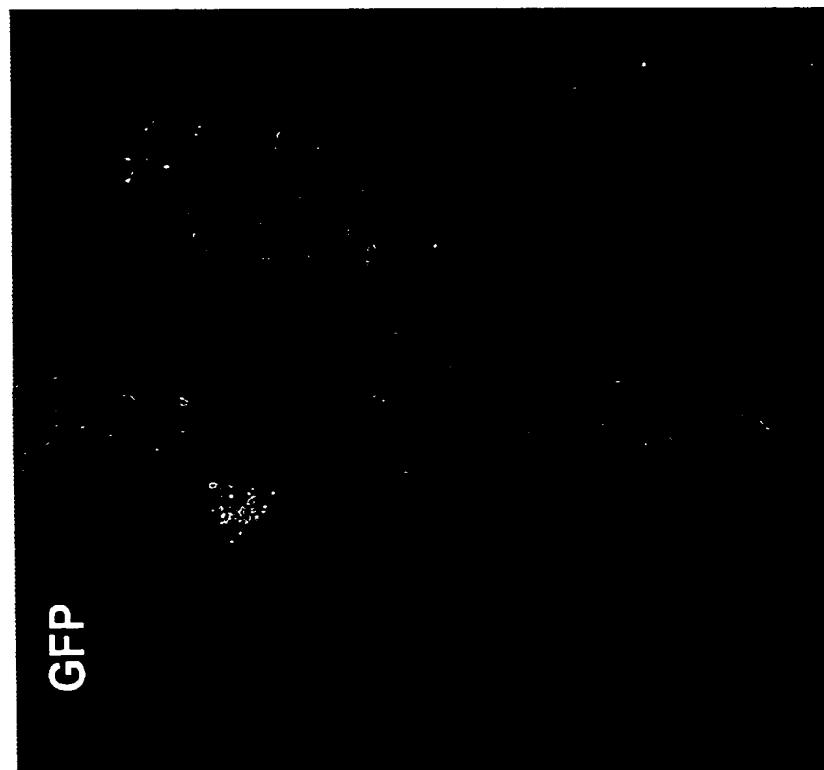
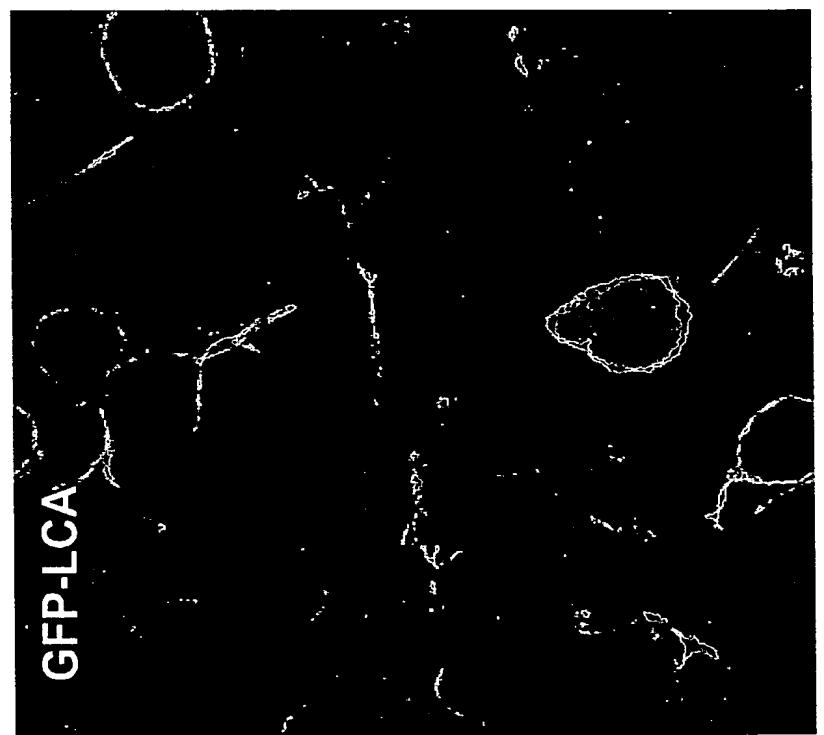


FIG. 21

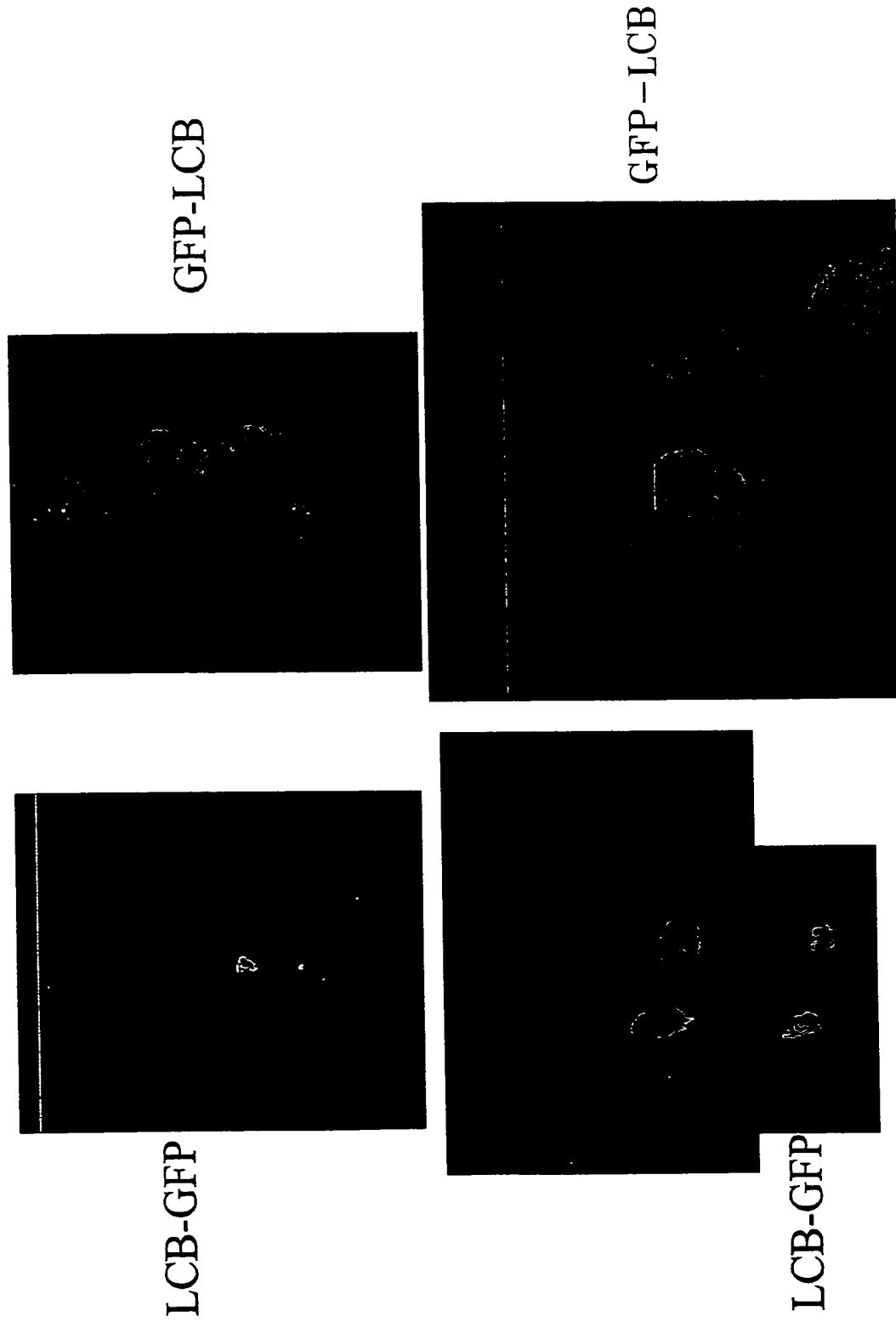


FIG. 22

Confocal

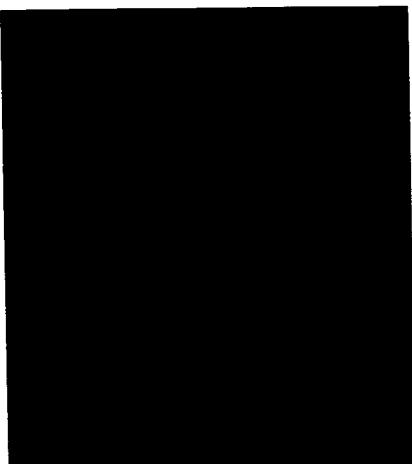
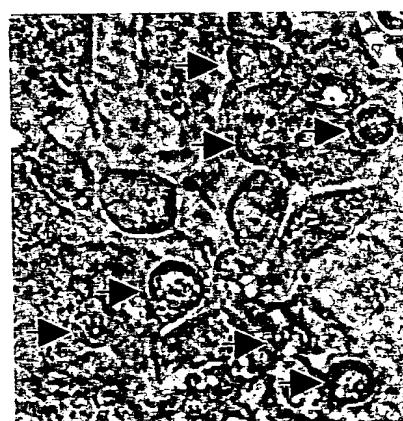
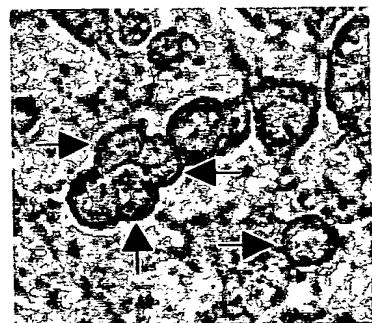
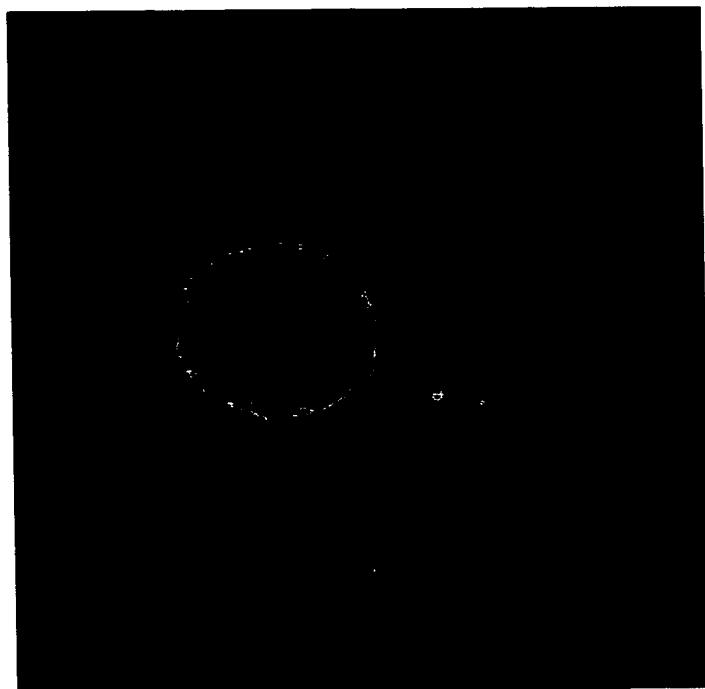


FIG. 23

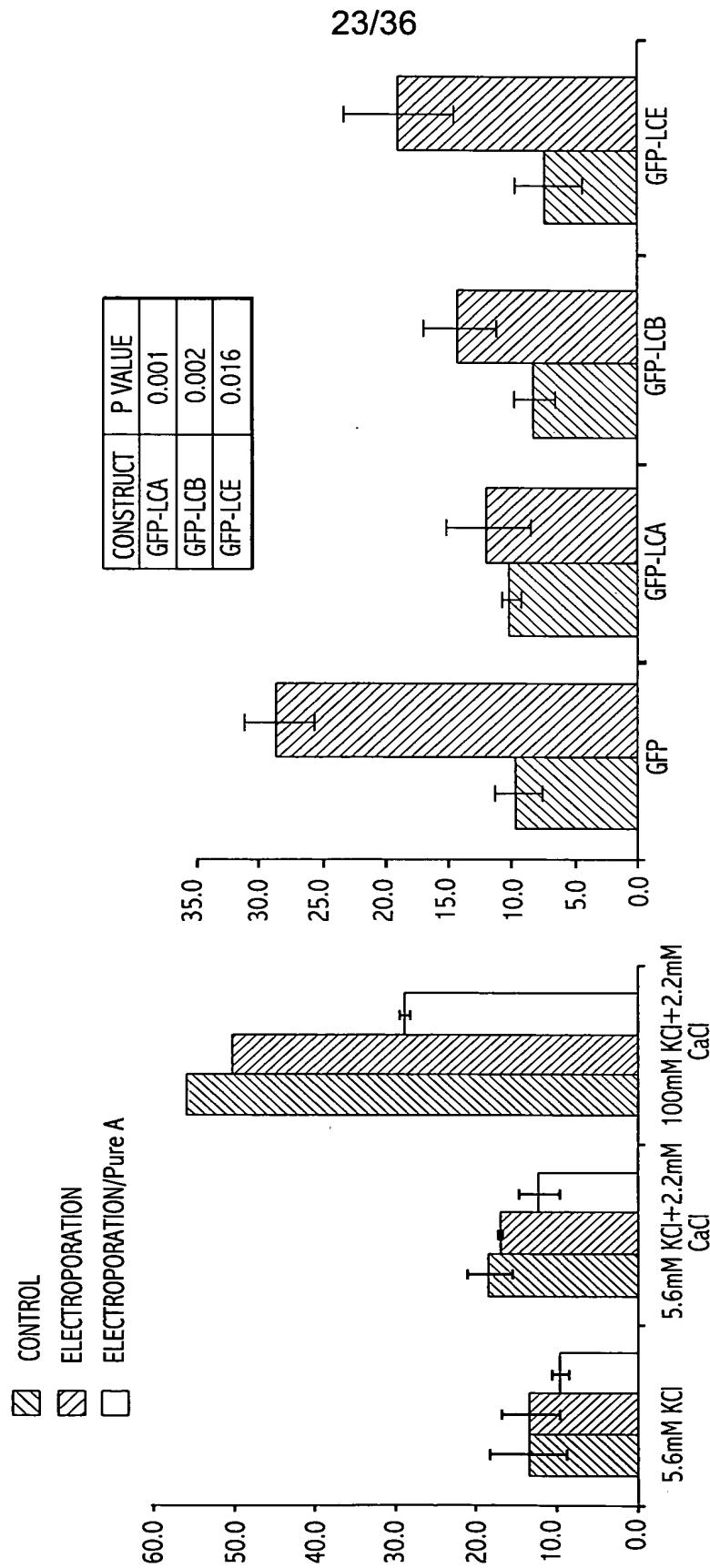
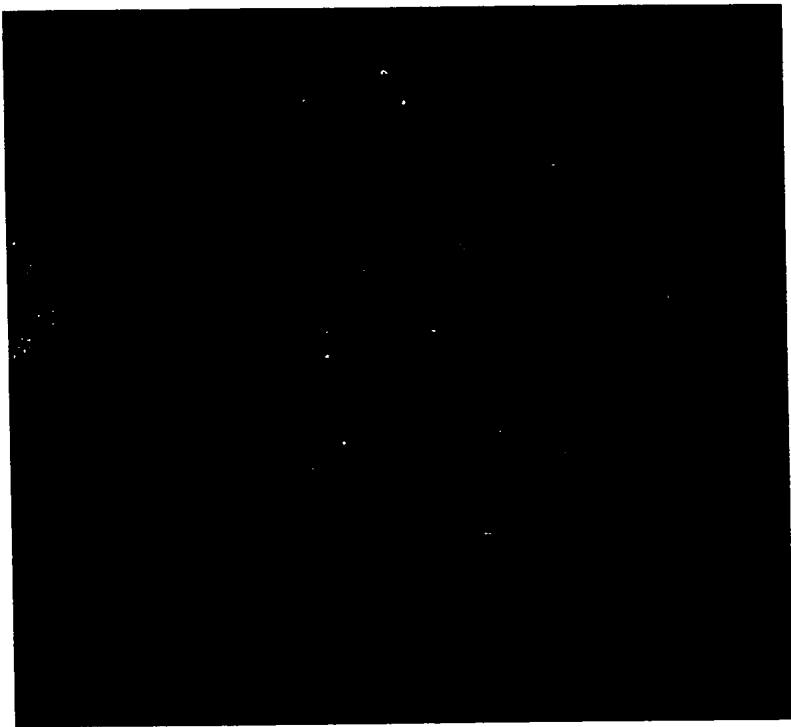
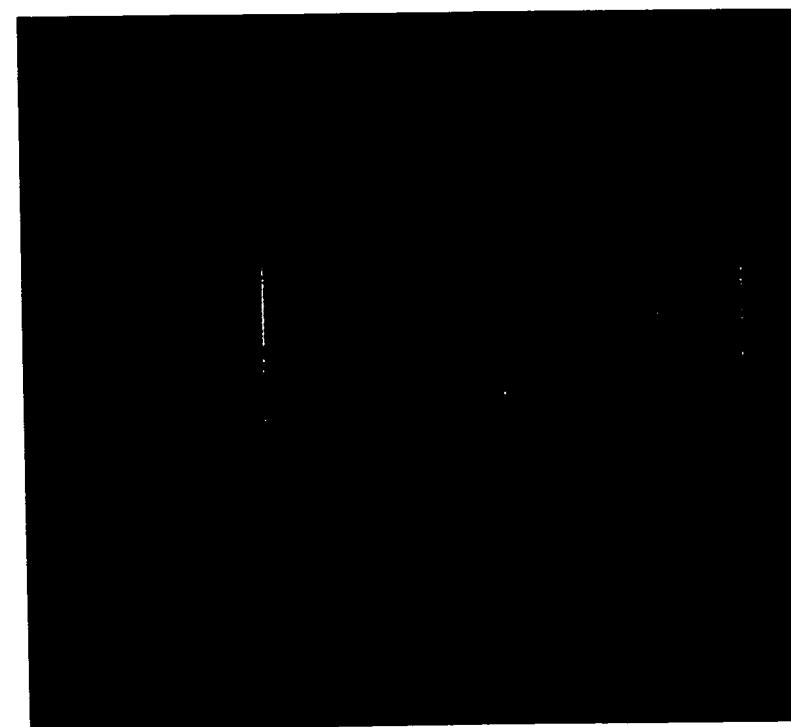


FIG. 24A

FIG. 24B



HEK293T



HeLa

FIG. 25

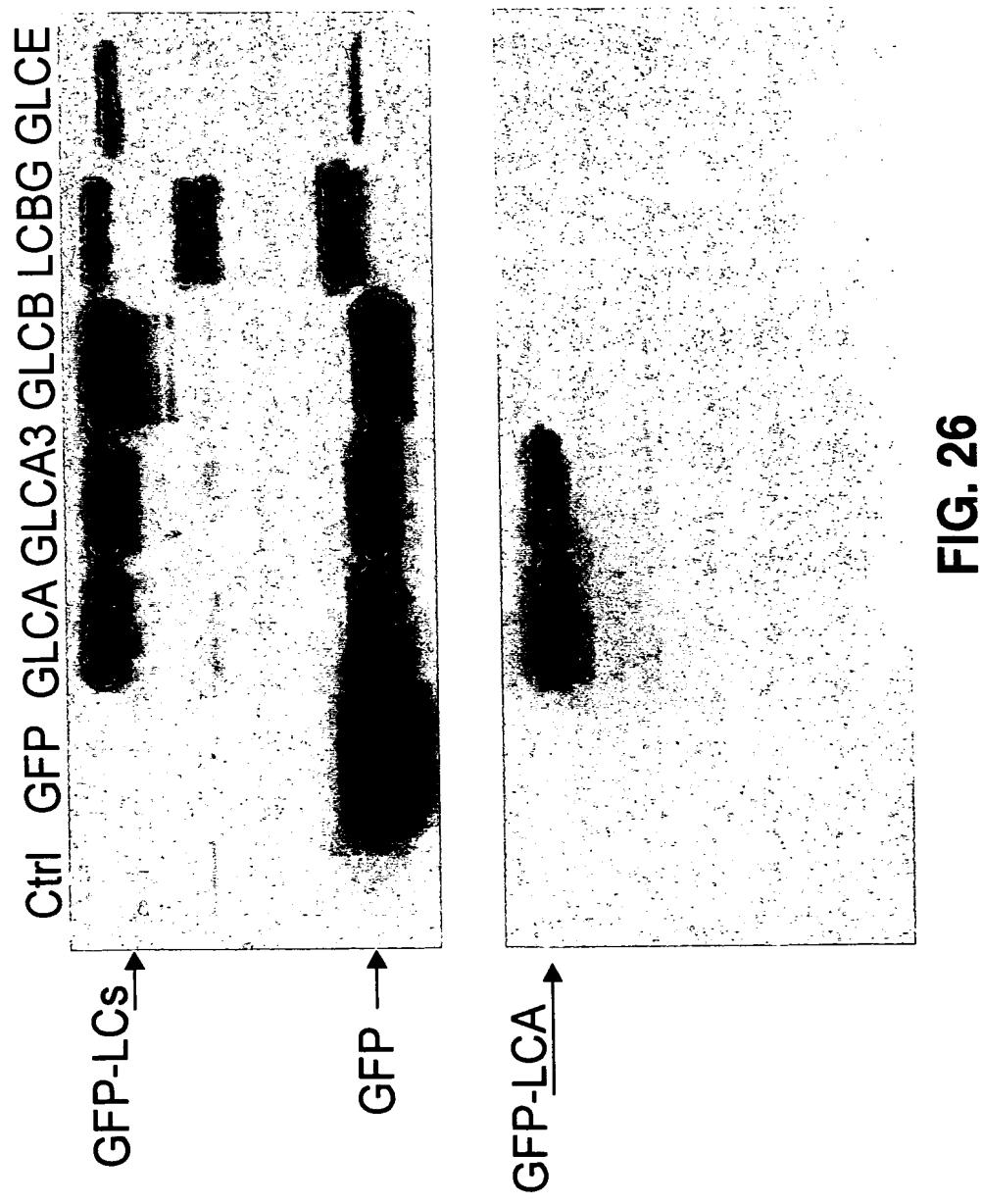


FIG. 26

FIG. 27

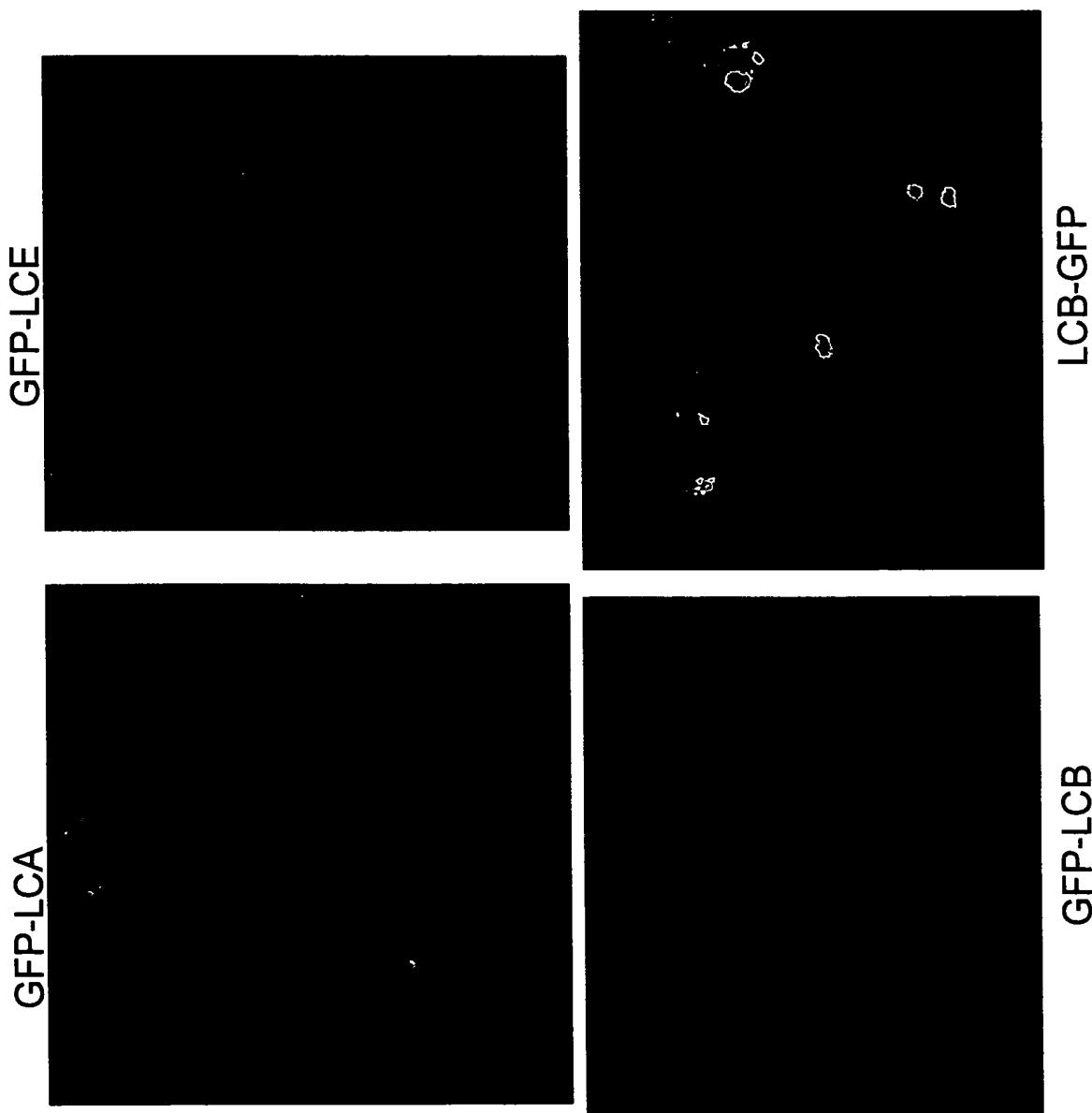
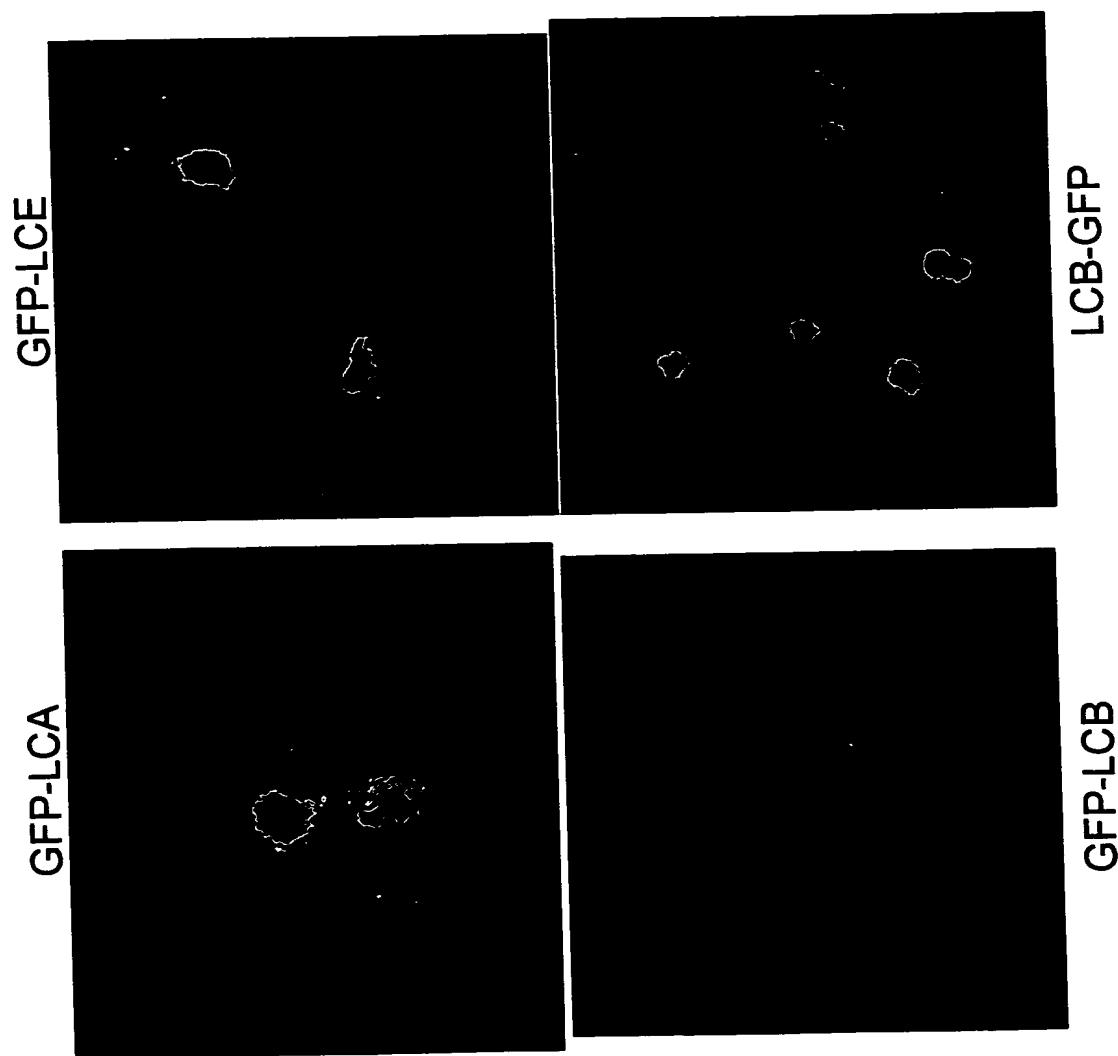




FIG. 28

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FIG. 29



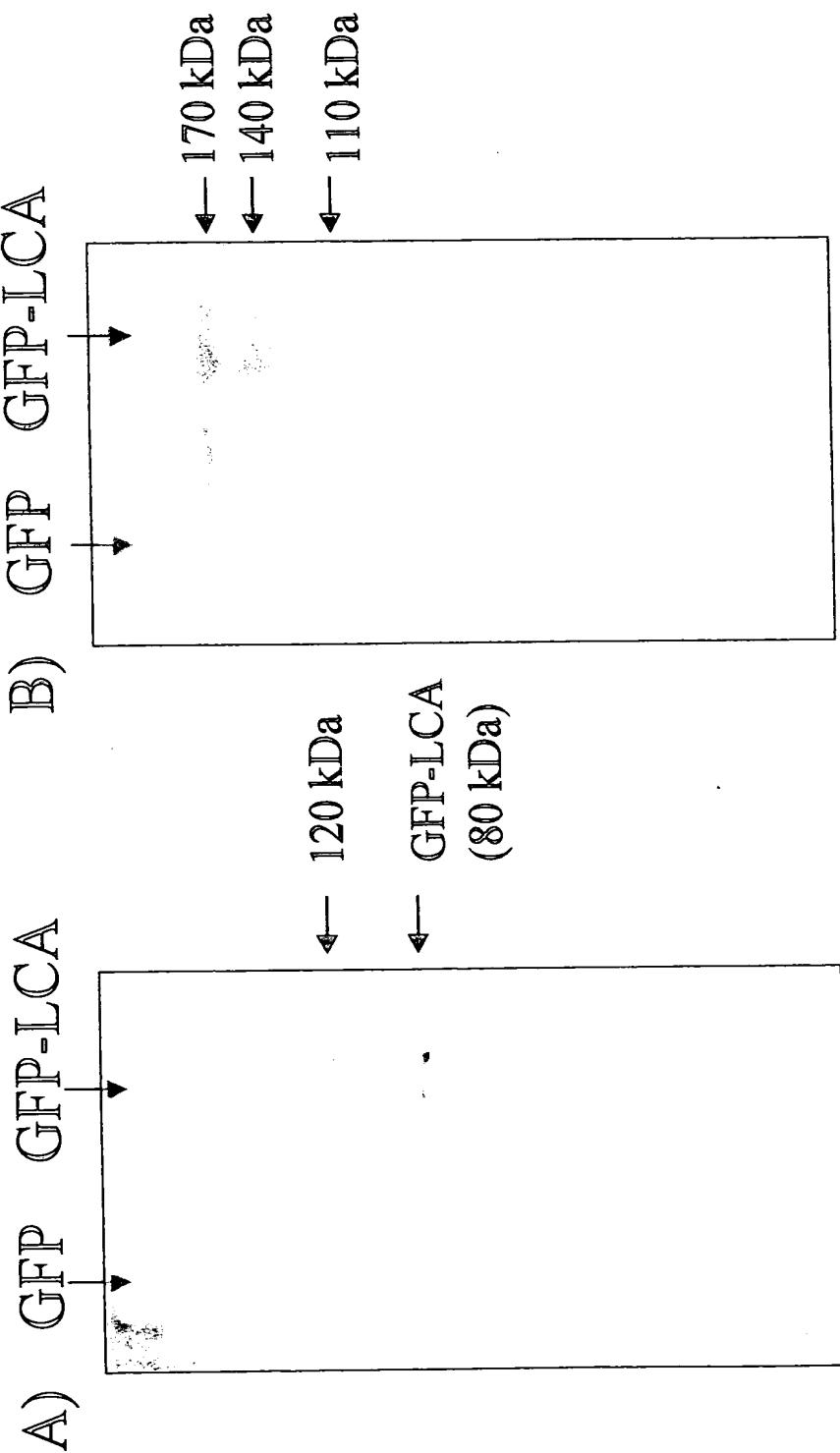


FIG. 30

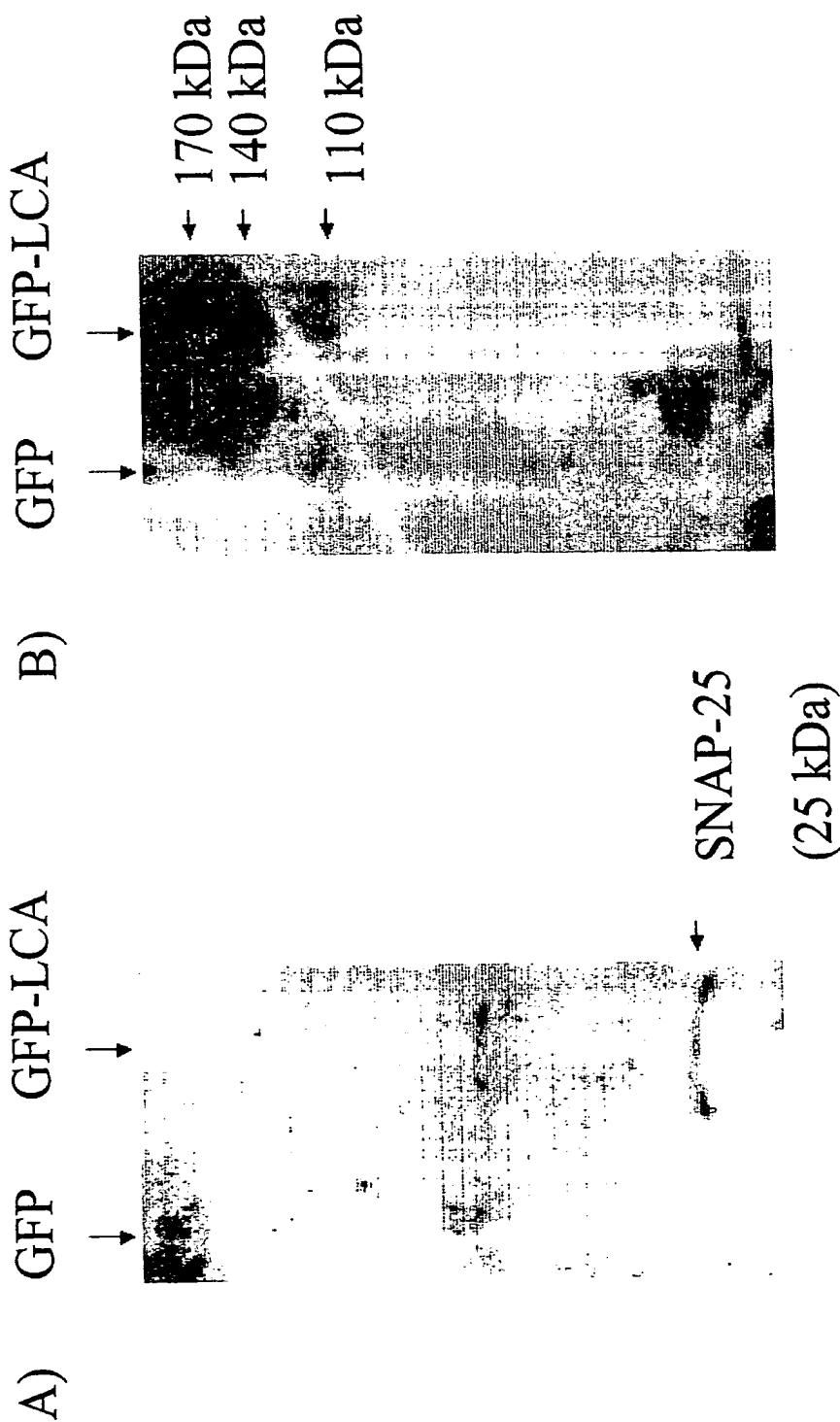


FIG. 31

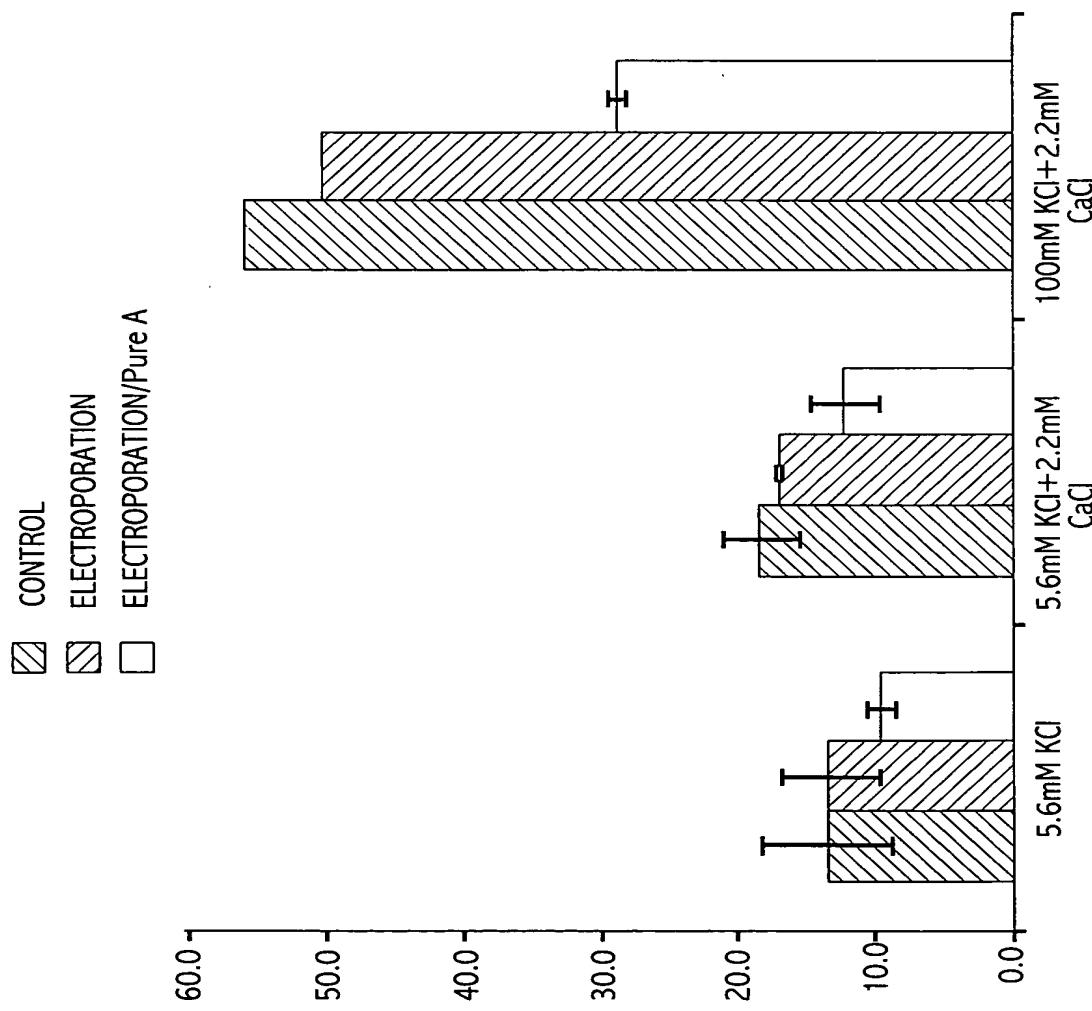


FIG. 32

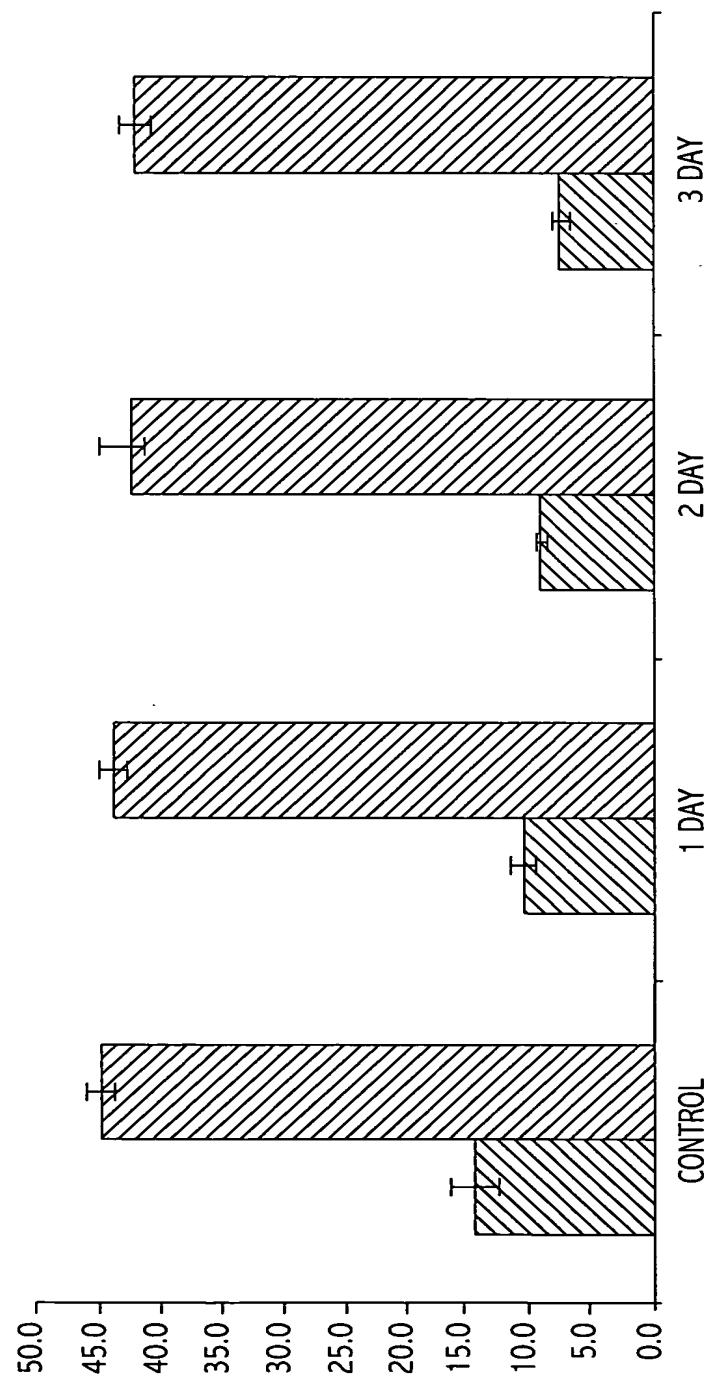


FIG. 33

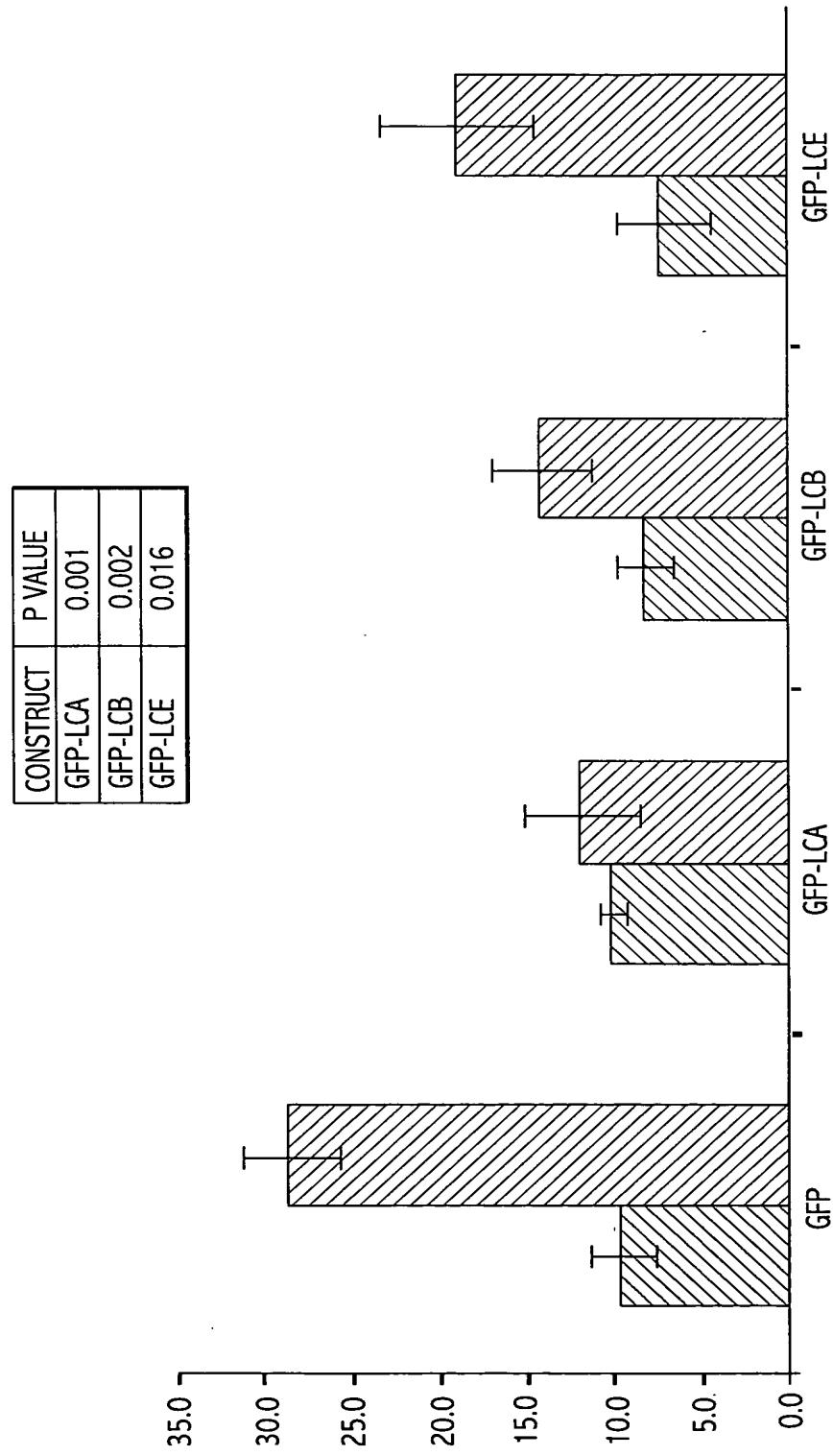
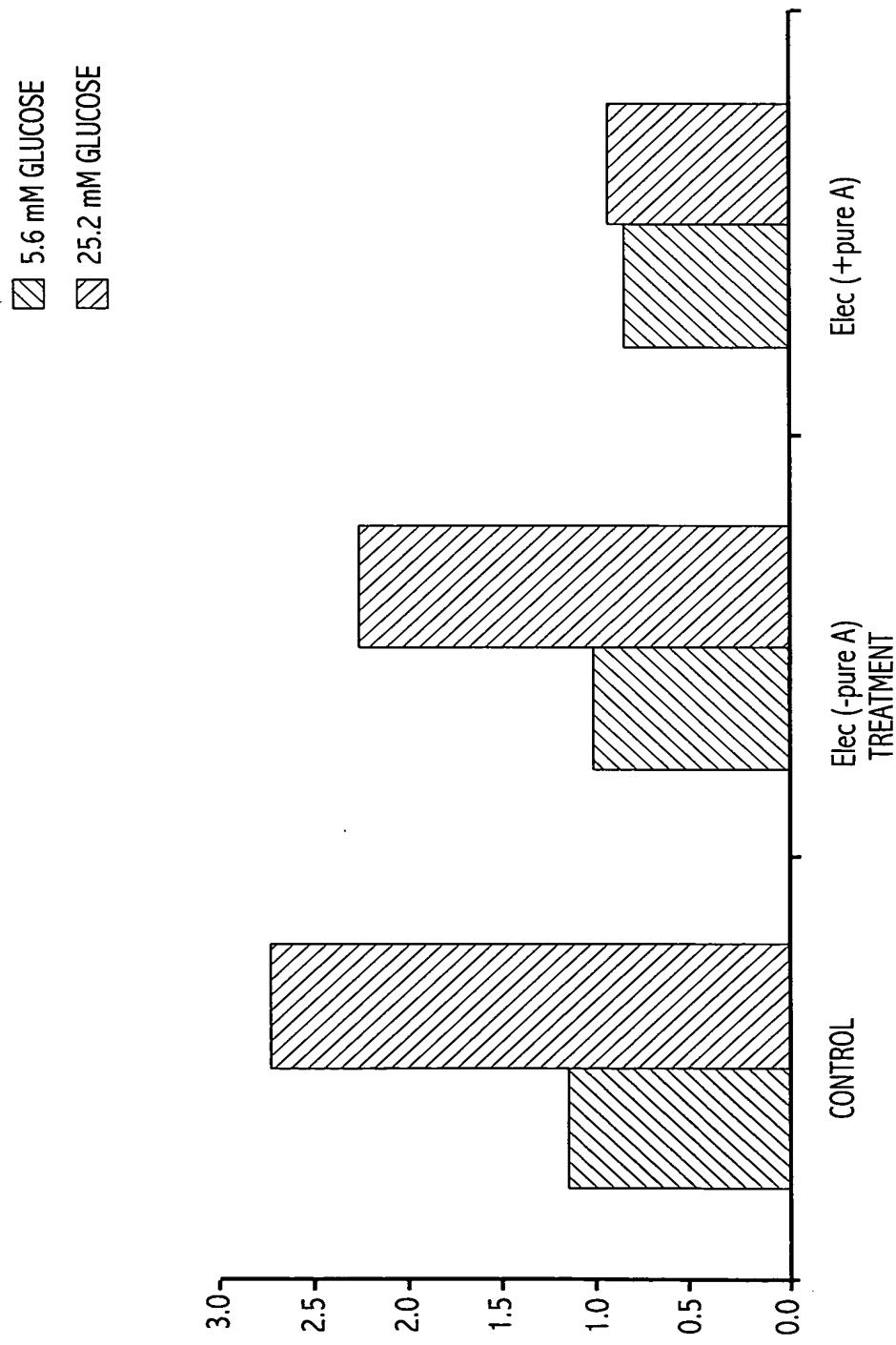


FIG. 34

**FIG. 35**

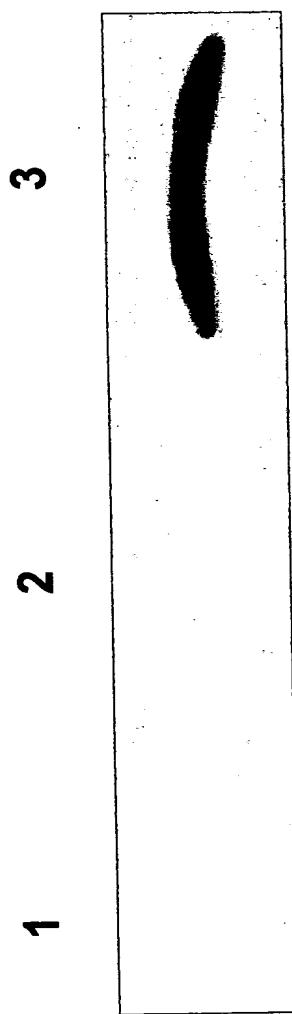


FIG. 36

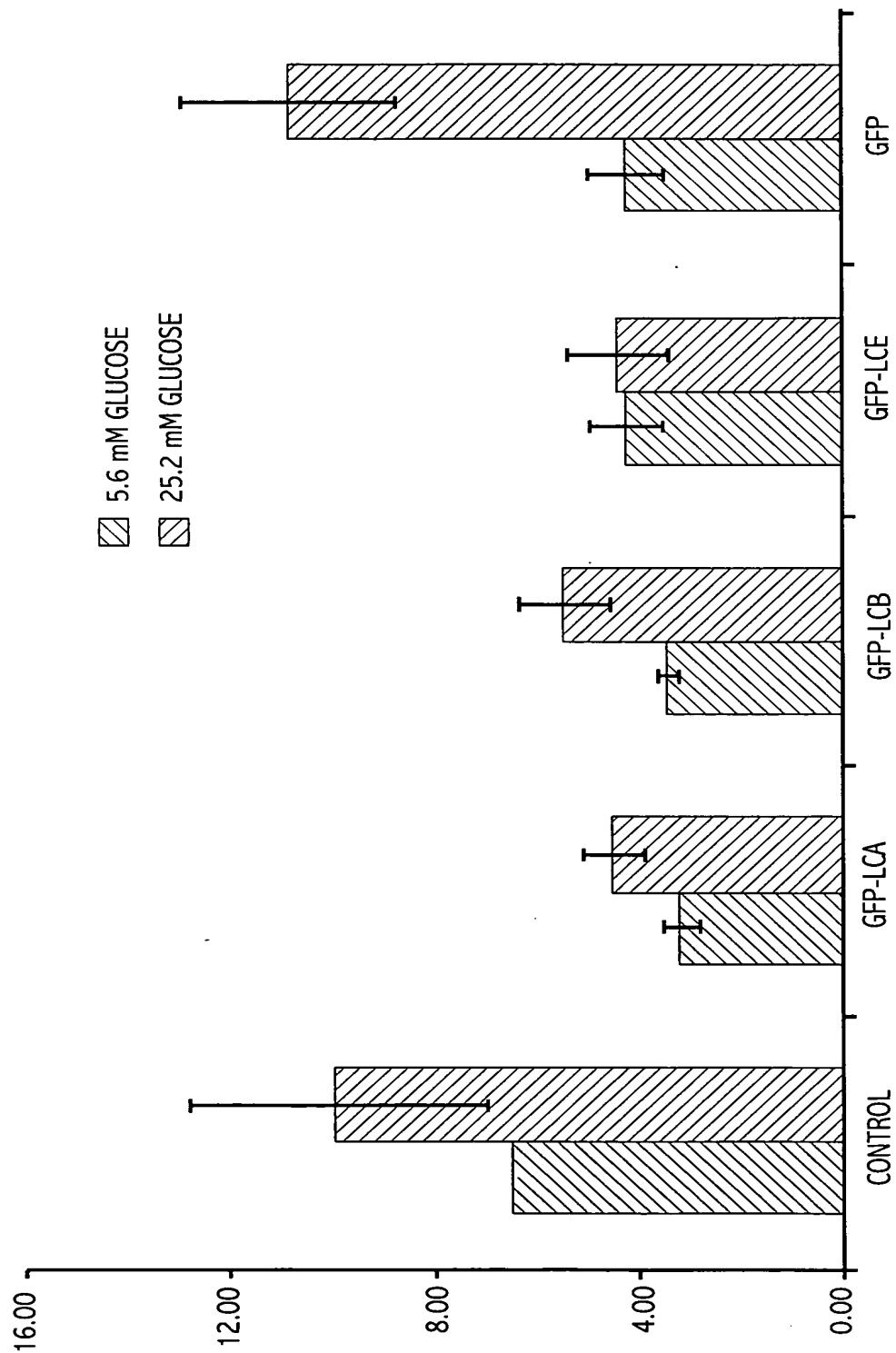


FIG. 37